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ABSTRACT

EVALUATION OF THE NITROUS OXIDE METHOD
FOR THE DETERMINATION OF CORONARY BLOOD FLOW

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FOR THE DETERMINATION OF CORONARY BLOOD FLOW*

The purpose of this study was to evaluate the nitrous oxide method for the determination of coronary blood flow. The method was compared with the Fick method, which is considered the gold standard for this purpose. The results of the study are presented in the following sections.

A comparison was made of the results obtained by the nitrous oxide method and the Fick method. The results of the nitrous oxide method were compared with the results of the Fick method. The results of the nitrous oxide method were compared with the results of the Fick method. The results of the nitrous oxide method were compared with the results of the Fick method.

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Based on data from a study of the left heart (the left heart injected with known amounts of dye, and the dye was observed in the coronary arteries), the authors have determined that the nitrous oxide method is accurate for the determination of coronary blood flow. The results of the study are presented in the following sections.

from

Medical Department Field Research Laboratory
Fort Knox, Kentucky
30 August 1949

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ABSTRACT

EVALUATION OF THE NITROUS OXIDE METHOD FOR THE DETERMINATION OF CORONARY BLOOD FLOW

OBJECT

The employment of the nitrous oxide procedure utilizes the animal in a state more closely approximating normal than any method for coronary blood flow measurement heretofore reported. The importance of establishing its validity is apparent.

A comparison has been made of the simultaneous values for left coronary blood flow in the open-chest, anesthetized dog as measured indirectly by the nitrous oxide method, and directly by an optically recording rotameter. The inflow side of the rotameter was connected to a carotid artery, the outflow connection was tied within the left coronary ostium. The dog breathed a nitrous oxide mixture, and saturation and desaturation curves were established by blood samples drawn from the rotameter and from a catheter within the coronary sinus.

RESULTS AND CONCLUSIONS

Based on dyed heart weight (the left heart injected with Evans Blue, ante mortem or post mortem), the nitrous oxide values differed maximally from the rotameter measurements by +50 to -17 per cent in 15 comparisons on 10 dogs. In 11 comparisons in 9 dogs, a somewhat better correlation (+18 to -10 per cent) was found between the two methods when the rotameter flow measurements were based on left heart weight (left ventricle, left atrium and total septum). The nitrous oxide values exceeded the rotameter flow values in 10 of 15 and 9 of 11 comparisons when based on dyed and weighed myocardium, respectively.

Since tests have established that the maximum error with the rotameter approximates 5 per cent, these sizeable differences between the values obtained with the two methods could arise either from the inaccuracy of the nitrous oxide method as applied to the anesthetized dog, or from inability to determine accurately the weight of the myocardium fed by the left coronary artery. These possibilities are being investigated.

RECOMMENDATIONS

None

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EVALUATION OF THE NITROUS OXIDE METHOD FOR THE DETERMINATION OF CORONARY BLOOD FLOW

I. INTRODUCTION

A method whereby the myocardial blood flow could be measured in the intact animal and man would be highly desirable, not only in establishing normal values, but as a supplement to other methods of studying the heart in the diseased state. The nitrous oxide method for measuring blood flow as devised by Kety and Schmidt has been employed for the determination of cerebral blood flow in unanesthetized man (1-8), and for measuring the myocardial blood flow in anesthetized dogs (9), unanesthetized dogs (10), and humans (11). This procedure approaches this aim more closely than other procedures reported. The importance of establishing the validity of the method is thus apparent. In brief, the Fick principle upon which the method depends, is as follows: The blood flow per unit of time through an organ is equal to the amount of a substance taken up by that organ in a given time divided by the difference in concentration of the substance in the arterial blood supply and venous drainage of the organ in the same time period. In the nitrous oxide method, the denominator in the Fick equation is found by computing the integrated difference between the concentration of nitrous oxide in arterial (samples drawn from any artery) and venous (samples drawn from vein draining the tissue studied) blood during the period of equilibration with low concentrations of respired nitrous oxide. The concentration of the gas in the tissue at the time of equilibrium (the numerator in the Fick equation) is unobtainable directly in the intact animal or man, and is assumed to be equal to the product of the venous concentration of the gas (after equilibrium is established) and a partition coefficient (unity in the case of brain (12) or heart (9)). When the equation is multiplied by 100, units for blood flow values are obtained which, in the case of the heart, are expressed as cc. of blood flow per 100 gm. of myocardium per minute.

Eckenhoff et al. (9) found satisfactory agreement between left coronary blood flow values obtained with the nitrous oxide method and values obtained simultaneously with direct measurements made with the bubble flow meter (13) in the anesthetized dog. For the direct values, the arterial inflow through the peripheral end of a cannulated circumflex or anterior descendens branch of the left coronary artery was measured. The flow in cc./100 gm. of left ventricle was determined by dividing the measured flow by the quantity of heart tissue stained when Evans Blue dye was injected into the cannulated artery, either ante mortem or post mortem. The conclusion was reached that gross contamination of coronary sinus blood from the right atrium did not exist because, (1) a 0.1 per cent solution of Evans Blue dye injected into the inferior vena cava or right auricle did not appear in the coronary sinus blood before recirculation through lungs and heart (as sampled through a contained catheter (14)); (2) the nitrous oxide saturation curve of coronary sinus blood was similar from dog to dog. However, no comparisons of the direct and nitrous oxide methods were reported in which venous blood samples for the nitrous oxide

curve were taken from an indwelling catheter. Actually, the venous samples for the nitrous oxide procedure were taken from a cannula tied into the great cardiac vein (15).

The present investigation is an endeavor to evaluate the nitrous oxide method for measuring left coronary artery blood flow, (1) when venous blood samples for the nitrous oxide procedure are withdrawn from an intravenous catheter introduced from within the venous system and lying freely in the coronary sinus; and (2) by comparing it with direct measurements of the total left coronary artery inflow continuously recorded with an optically recording rotameter (16, 17) during the test period.

II. EXPERIMENTAL

A. Apparatus, Methods and Procedures

Mongrel dogs of either sex weighing 15 to 25 kgm. were anesthetized with sodium pentobarbital, 20 mgm./kgm. given intravenously. A cannula for infusion purposes was placed in the right femoral vein and kept patent by means of a slow drip of saline.

A No. 6 $\frac{1}{2}$ -8 tapered intravenous catheter (14) was connected to a saline source containing 30 units of heparin/1000 cc. and inserted through a branch of the left external jugular vein into the superior vena cava. A slow flow of saline-heparin solution was established through the catheter to prevent obstruction by blood clots. Under fluoroscopy, with the animal in the right anterior oblique position, the catheter was guided into the coronary sinus. The catheter was usually pushed well into the great cardiac vein to prevent its possible escape into the inferior vena cava during subsequent manipulations of the animal.

The dog was then placed on his right side with a sandbag under the thorax. Under artificial respiration through an endotracheal tube with an inflated balloon, the chest was opened and portions of the 7th-11th left ribs were resected. The pericardium was opened by a cross-incision after ligating any large vessels on its surface, and a cradle was formed by anchoring it to the chest wall with hemostats. The left auricle was retracted from the field by means of a suture tied to a small portion of its border. Retraction by hemostats on the fatty tissue inside the curvature of the pulmonary conus brought the location of the left coronary artery into view. The artery was carefully cleaned of all connective tissue from its origin to the region of bifurcation into the descendens and circumflex branches and a No. 00 silk suture passed around it.

Following intravenous anticoagulants (5 per cent pontamine fast pink 3 cc./kgm. and 15 units of heparin) a cannula with a three-way stop-cock attachment was inserted centrally into the right common carotid artery and connected by means of a short piece of rubber tubing with a 5 mm. lumen to the afferent side of an optically recording rotameter having a flow range of 0 to 200 cc. (17).

The rotameter arrangement is illustrated in Figure 1. From the efferent side of the rotameter, a 12-inch rubber tube leads to the coronary cannula. The coronary cannula consists of a 12-cm. length of 4-mm. thin wall brass tubing. To one end, which has been cut off diagonally, a short brass lip is soldered at an angle of approximately 135 degrees. A right angle bend is made in the same plane as the diagonal tip so that the distance from tip to right angle is 4 cm. To prevent too deep an insertion of the cannula into the coronary artery and ultimate ventricular fibrillation, the tip is 2 mm. or less in length. A rubber tubing shunt is included in the system whereby the blood flow can be shunted around the rotameter as desired. This affords a convenient means of establishing the rotameter zero before and after a flow recording and for calibrating the rotameter in situ. The system is filled with saline, and 1 cc. of heparin is placed in the chamber above the rotameter float.

The coronary cannula was inserted through an artificial opening into the brachiocephalic artery about 2 cm. from its origin and pushed down the ascending aorta to the region of the left coronary ostium. The saline in the system was replaced with blood by opening a side tube and letting blood flow through the tubing, first from the right common carotid artery and then from the aorta. Besides removing the saline and any bubbles that may have been present, this served the additional purpose of verifying the patency of both the carotid and coronary cannulae. The blood was returned to the animal through the intravenous infusion apparatus.

The coronary cannula was guided by palpation and visualization of the prominence of its tip into the left coronary artery where it was tied as closely as possible to the aorta with the previously placed No. 00 thread. The sandbag and all hemostats were removed following the cannulation. The three-way stopcock on the carotid cannula was connected with plastic tubing to a mercury manometer and a Gregg optical manometer (18) for recording mean blood pressure.

The catheter in the coronary sinus was then palpated for location and withdrawn or further inserted so that its tip lay 3-5 cm. inside the sinus. In no experiment after final placement of the catheter was there observable distention of the great cardiac vein.

All experiments were done in open-chest animals with positive pressure respiration. Irregularities in cardiac rhythm occasionally developed an hour or so after cannulation, but in general, the animals remained in an acceptable condition up until the time of sacrifice. After adequate technique had been established, the experiments were usually completed within 3 hours.

In some experiments, spot rotameter calibrations were made before the nitrous oxide run, using blood withdrawn from the animal and checked with previous calibration curves made with a solution of methyl cellulose (specific viscosity, 4). In all experiments, a rotameter calibration curve was established with the animal's own blood after the test period. The spot calibration points did not vary from the final curve by more than 5 per cent.

Except for the positive pressure respiration which was used in the present investigation, the nitrous oxide procedure for measuring blood flow was similar to that of Kety and Schmidt as adapted for the coronary circulation by Kelenhoff *et al.* The gas mixture consisted of 15 per cent nitrous oxide, 21 per cent oxygen, and 64 per cent nitrogen. Venous samples were drawn from the tapered catheter lying freely in the coronary sinus. A catheter of identical capacity with that used for drawing the venous samples was attached to the efferent tube of the rotameter near the coronary cannula and served as a means for drawing the arterial blood samples (Fig. 1). The blood samples of 6 ml. each were taken as follows: A blank was first drawn from either artery or vein; 1A and 1V were drawn evenly from the beginning of nitrous oxide insufflation to the end of 1 minute; 2A and 2V from 1 min. 5 sec. to 1 min. 35 sec.; 3A and 3V from 2 min. 45 sec. to 3 min. 15 sec.; 4A and 4V from 4 min. 45 sec. to 5 min. 15 sec.; 5A and 5V from 9 min. 45 sec. to 10 min. 15 sec. Samples for a desaturation curve were drawn at identical times except in one case in which 5A and 5V were taken at 7 min. 45 sec. to 8 min. 15 sec. The blood samples were collected in 10 ml. oiled syringes through manifolds (2) connected to the arterial and venous catheters.

All gas analyses were made with the Van Slyke-Neill manometric apparatus by the method of Orcutt and Waters (19) as modified by Kety (20). Most of the duplicates agreed within 0.02 volume per cent. In a few experiments, duplicates were not made for all samples.

To facilitate the change from air inflation to nitrous oxide mixture inflation, two Dann respirators were attached to the tracheal tube by means of a brass T-tube and short pieces of pressure tubing. One respirator was connected to an air pressure source, the other was attached to the tank of the special gas mixture. The respirator not in use was isolated from the animal by clamping the tubing between it and the tracheal tube. An escape vent in the tubing between the clamp and respirator prevented excessive pressure in the tube and allowed the system to be filled with the gas up to the clamp. The respirators were so adjusted as to have a similar depth and rate. Approximately thirty seconds before nitrous oxide breathing was to begin, the nitrous oxide tank regulator was opened. At zero time, the clamp was quickly released from the nitrous oxide tube and, simultaneously, another clamp was closed in a similar position on the air tube.

If a nitrous oxide desaturation curve was desired, the animal was allowed to breathe the gas mixture for approximately 15 minutes, after which the above procedure was reversed.

For comparison of the direct and nitrous oxide methods, a continuous photokymographic record of the direct flow and mean blood pressure was made throughout the time of the nitrous oxide procedure as well as for the preceding minute of control flow. The camera was 37 centimeters from the recording meter and the photographic paper was fed at the rate of 15 cm. per minute. A rotameter zero (recorded) was established before and after each test period.

The average left coronary inflow directly measured was computed by planimetric integration of the recorded rotameter deflection covering

the period of comparison of the two methods. The average deflection was then referred to the rotameter calibration curve where it could be read as cubic centimeters of blood flow. Since the quantity of blood drawn from the efferent tube of the rotameter for arterial samples and clearing made the recorded flow greater than the amount actually going through the left coronary artery, this volume was subtracted from the 10-minute (or 5-minute) direct flow value as measured (i.e., in the case of a 10-minute record, if 30 cc. were withdrawn for arterial blood samples and a total of 7 cc. for clearing, then 3.7 cc. were subtracted from the recorded direct flow value calculated as cc./min.).

Flow per 100 gm. for the direct method was determined on the basis of left heart weight and by weighing the amount of heart tissue (moist weight) taking up the blue stain following the injection of 0.5 per cent Evans Blue dye through the cannula into the left coronary artery. The injection was accomplished in different experiments in one of three ways: (1) by injecting the dye at a low pressure into the blood flowing into the left coronary artery while the animal was alive and then immediately inducing fibrillation by an inductorium; (2) by injection in situ post mortem, or (3) by removing the heart (the left coronary cannula remaining in place) cannulating the right coronary artery and injecting contrasting dyes simultaneously and at equal pressures of 100-150 mm. Hg into the right and left coronary arteries.

B. Results

Differences in the percentage of tissue stained by the 3 methods appeared insignificant as can be seen from Table 1 which includes donor dogs as well as experimental animals.

Figure 2 shows a nitrous oxide curve typical of those obtained in hearts with large coronary flows. Figure 3 shows a reproduction of a representative section of the photokymographic record from the same experiment. The results of 15 comparisons in 10 animals are presented in Table 2. The first two experiments can be considered significant only insofar as they compare with some of the others. Since the cannulated left coronary artery was not injected with dye in these 2 observations, it is not known whether any of its major branches were partially occluded. If one of the larger branches is blocked by the cannula, exceptionally low values will be computed when the directly measured left coronary blood flow (cc./100 gm.) is based on the left heart weight. Experiments EE687 through EE690 are those in which the septum was partially or completely unstained following the injection of dye into the left coronary artery. Three other observations, two of which showed good agreement between the two methods, have been excluded from the table either because of distortion of the nitrous oxide curve, deviation in the rotameter calibration curve of more than 5 per cent, or discrepancies between the duplicates of the nitrous oxide analyses.

Based on dyed heart weight, the nitrous oxide values differ maximally from the rotameter measurements by +50 to -17.5 per cent. In EE690, the percentage variation between the two methods is about twice as great as in any other comparison. A better correlation is found between

the two methods when the rotameter flow measurements are based upon left heart weight (left ventricle, left atrium, and total septum).

The nitrous oxide flow values exceed the rotameter flow values in 10 of 15 and 9 of 11 comparisons when based on dyed and weighed myocardium, respectively.

III. DISCUSSION

These observations show that a considerable difference exists between the left coronary flow as determined with the rotameter and the nitrous oxide method. The significance of this difference remains to be established. Critical tests have established that the total coronary flow values with the rotameter have a maximum error of approximately 5 per cent. This raises the question whether the nitrous oxide method as applied to the anesthetized dog is in error or whether a substantial error is introduced into the rotameter flow values/100 gm. left heart through inability to determine accurately the weight of myocardium actually nourished by the left coronary artery.

Based on these two possibilities, various sources of error can be thought of, but at present no choice can be made between them. The generally higher flow values found with the nitrous oxide method could be related to different factors.

If a time lag exists between the arterial blood flowing through the recording meter and that which flows through the intact coronary arteries, and if there exists an overlap of these sources to the area of the myocardium whose venous drainage is being studied, the venous nitrous oxide concentration will rise too rapidly and the flow calculated by the nitrous oxide method will be too high. In the experiments of Eckenhoff et al. (9), this might be a source of error since a time lag approximating one minute existed between the arterial blood passing through the bubble flow meter and that flowing through the intact coronary artery, and a major branch of the left coronary artery which drains into the coronary sinus was not cannulated. However, in the present investigation, this error from arterial overlap is presumably less, for the time required for methyl cellulose (sp. visc., 4) to go from the carotid cannula to the coronary cannula through the rotameter system approximated 1-1/2 seconds at 70 mm. Hg mean aortic pressure; the left coronary artery was cannulated and the contribution of the right coronary artery to coronary sinus drainage is considered insignificant (21). However, it is conceivable that with a low blood pressure and a low blood velocity through the rotameter system, a considerable error might be introduced in the nitrous oxide method if the right coronary overlap was extensive.

Overinjection of the heart with dye will cause the rotameter values per 100 grams of heart to be too low.

Similarly, the lower nitrous oxide values could be related to (1) understaining of the myocardium, (2) admixture of coronary sinus blood with blood which does not drain the myocardium and which has a lower nitrous oxide concentration, such as that in the right atrium or that draining from the fatty tissue of the heart.

IV. CONCLUSIONS

A sizeable difference can exist between the left coronary flow as determined with the rotameter and the nitrous oxide method. Since the maximum error with the rotameter approximated 5 per cent, the discrepancy could arise from the fact that the nitrous oxide method as applied to the anesthetized dog is in error and/or that the weight of the myocardium actually nourished by the left coronary artery cannot be precisely determined and hence the rotameter flow values/100 gm. left heart/minute are not accurately calculated. At present no choice is possible between these two possibilities.

V. RECOMMENDATIONS

None.

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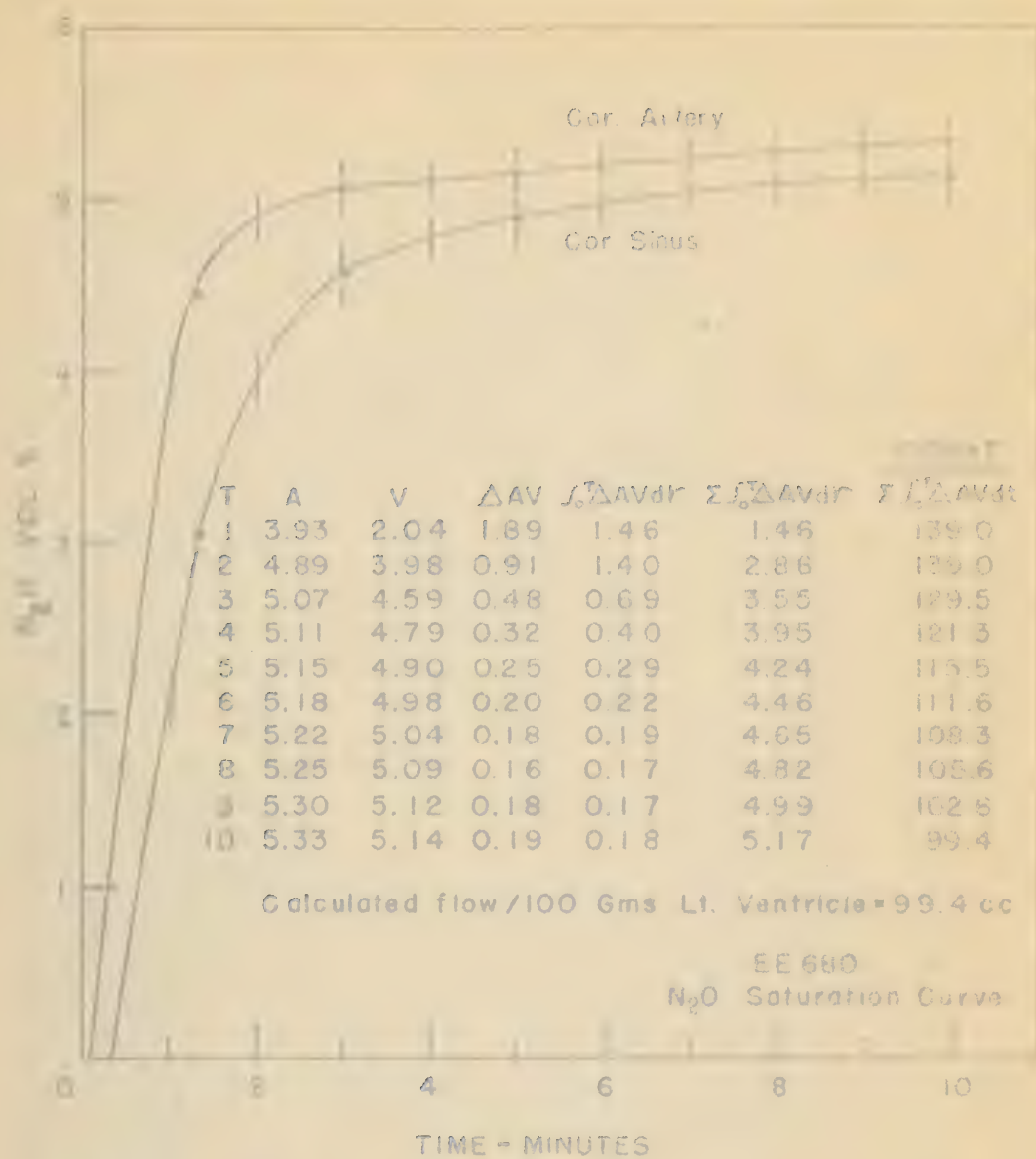


FIG. 5: GRAPH OF NITROUS OXIDE SATURATION CURVE FROM SAME EXPERIMENT AS IN FIG. 2

TABLE I
PERCENTAGE OF HEART STAINED FOLLOWING INJECTION OF LEFT CORONARY ARTERY

EXP. NO.	BODY WT. KGM.	HEART WT. GM.	HEART WT. % OF BODY WT.	LEFT COR. ART. INJECTED WITH 0.5% EVANS BLUE DYE		LEFT HEART ** GM.	LEFT HEART % OF TOTAL HEART	COMMENTS
				DYED HEART GM.	UNDYED* HEART GM.			
EE672	17	117.6	0.69	96.9	20.7	82.0	87.2	LEFT COR. ART. INJECTED POST MORTEM.
EE674	16	177.8	1.1	148.3	29.5	83.0	128.5	LEFT COR. ART. INJECTED POST MORTEM.
EE676	16	137.3	0.86	113.0	24.3	82.0	103.8	LEFT COR. ART. INJECTED POST MORTEM.
EE678	10	87.3	0.87	65.0	22.3	77.0	67.3	RIGHT AND LEFT COR. ART. INJECTED AT EQUAL PRESSURES POST MORT.
EE679	12	102.6	0.85	80.5	22.1	79.0	70.8	RIGHT AND LEFT COR. ART. INJECTED AT EQUAL PRESSURES POST MORT.
EE679	10	76.8	0.77	63.8	13.0	83.0	59.1	RIGHT AND LEFT COR. ART. INJECTED AT EQUAL PRESSURES POST MORT.
EE680	20	149.2	0.74	117.7	31.5	78.0	110.0	RIGHT AND LEFT COR. ART. INJECTED AT EQUAL PRESSURES POST MORT.
EE682	24	172.1	0.72	140.4	31.7	81.0	127.9	LEFT COR. ART. INJECTED ANTE MORTEM.
EE683	11	113.0	1.0	93.8	19.2	83.0	85.3	RIGHT AND LEFT COR. ART. INJECTED AT EQUAL PRESSURES POST MORT.
EE685	16	151.6	0.94	117.0	34.6	77.0	115.5	LEFT COR. ART. INJECTED POST MORT.
EE686	17	160.8	0.94	136.0	24.8	84.0	125.8	LEFT COR. ART. INJECTED ANTE MORT.
EE688	13	95.9	0.74	77.2	24.1	80.5	72.8	RIGHT AND LEFT COR. ART. INJECTED AT EQUAL PRESSURES POST MORT.
EE691	18	116.0	0.65	92.4	23.6	79.6	86.2	LEFT COR. ART. INJECTED ANTE MORT.
EE692	17	138.2	0.81	112.8	25.4	82.0	103.3	LEFT COR. ART. INJECTED ANTE MORT.
AVG	15.5	128.3	0.83	103.9	24.8	81.5	96.0	74.7

*UNDYED, OR THAT PORTION DYED BY INJECTING RIGHT CORONARY ARTERY.

** LEFT HEART = LEFT VENTRICLE, LEFT ATRIUM, AND TOTAL SEPTUM.

TABLE II
COMPARISON OF NITROUS OXIDE FLOW VALUES WITH ROTAMETER
FLOW VALUES BASED ON DYED HEART AND LEFT HEART

EXP. NO.	MEAN BLOOD PRES- SURE mm. Hg	LEFT CORONARY BLOOD FLOW PER MINUTE					COMMENTS	
		N ₂ O cc/100gm	ROTAMETER/DYED HEART cc/100gm	ROTAMETER/LEFT HEART*				
				N ₂ O-ROT. x100 ROT.	N ₂ O-ROT. x100 ROT.			
EE667	86	57.8 (S)			49.6	+ 16.2	SALINE 25 cc.; EPINEPHRINE 1.5 cc. OF 1:1000 DURING TEST PERIOD.	
EE668	105	87.6 (S)			74.4	+ 17.7		
EE674	75	79.6 (S)	63.0	+ 26.0	72.0	+ 10.5		
EE680	92	99.4 (S)	91.7	+ 8.4	98.2	+ 1.2		
EE682	77	73.3 (S)	72.0	+ 1.8	79.0	- 7.2		
EE685	100	94.4 (S)	80.6	+ 14.5	84.2	+ 9.7		
EE686	62	67.9 (S)	68.5	- 0.9	74.0	- 8.2		1 cc. CORAMINE; 50 cc. WHOLE BLOOD DURING TEST PERIOD.
EE687	65 60	64.4 (S) 47.2 (D)	78.0 52.0	- 17.5 - 9.2				
EE689	(88 (93	62.2 (S) 80.2 (D)	65.0 73.0	- 4.6 + 9.6				FIBRILLATION WITH REVIVAL PRIOR TO TEST PERIOD. N ₂ O (D) CALCULATED AT 8 MINUTES.
EE690	45 50	54.0 (S) 78.0 (D)	36.0 52.2	+ 50.0 + 50.0				
EE691	95 105	103.0 (S) 151.0 (D)	106.5 130.0	- 3.0 + 16.0	114.1 139.2	+ 9.7 + 8.4		
EE692	75 75	98.0 (S) 65.4 (D)	82.3 53.4	+ 19.5 + 22.5	89.4 58.3	+ 9.6 + 12.0		
AVG.	79	81.0** 88.6***	73.6		84.7			

** LEFT HEART = LEFT VENTRICLE, LEFT ATRIUM, TOTAL SEPTUM.

*** EXCLUDING THE FIRST TWO N₂O VALUES.

*** USING ONLY THOSE N₂O VALUES WHICH CORRESPOND TO ROTAMETER FLOW
VALUES BASED ON LEFT HEART WEIGHT.

S = N₂O SATURATION CURVE
D = N₂O DESATURATION CURVE

